

Demonstration of Reproductive Isolating Mechanisms in *Callosamia* (Saturniidae) by Artificial Hybridization

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Abstract. The isolating mechanisms in *Callosamia* form an elaborate array of complex and numerous ones including premating to postzygotic. The cross *C. securifera* ♂ X *C. promethea* ♀ is described for the first time, as well as a complex cross involving *C. angulifera* and *C. securifera*, these in addition to ten crosses reported earlier. Some of the results obtained are comparable to those achieved by other workers hybridizing other genera of Lepidoptera. Each isolating mechanism is discussed and interpreted. The relationship between speciation and isolating mechanisms is discussed also, with a review of some recent literature on this topic.

Introduction

The genus *Callosamia* Packard is a well-defined North American group of saturniid moths comprised of only three species: *C. angulifera* (Walker), *C. promethea* (Drury), and *C. securifera* (Maassen). Although generally considered to be "well-known", many questions remain unanswered about the phylogeny and ecology of these insects. Moreover, the barriers to hybridization in nature are much more complicated than simple differences in circadian mating behavior. Obtaining and rearing hybrids in the laboratory, and careful studies of natural populations can help elucidate these problems. The purpose of this paper is to figure and describe briefly artificial hybrids obtained since my report on ten earlier crosses (Peigler, 1977), and to discuss some of the evolutionary, ecological, and genetic aspects of the genus, with special reference to isolating mechanisms.

Descriptions of New Crosses

C. securifera ♂ X *C. promethea* ♀

Three broods of this cross were reared simultaneously. A brief account of each brood is given followed by a composite description of the stages based on all three broods. Table 1 contains numbers involved in the broods.

Brood 1 was the result of a hand-pairing using a male and female (both from wild-collected cocoons) from Berkeley County, South Carolina.

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Some larvae were reared on tuliptree (*Liriodendron tulipifera* L.) and some on sweetbay (*Magnolia virginiana* L.). Those fed on the former grew larger. Many larvae died of disease in the penultimate stadium.

Brood 2 was the result of hand-pairing a male from a wild-collected cocoon from Berkeley Co., S.C. and a reared female from Pine Grove, Schuylkill Co., Pennsylvania. Larvae were fed tuliptree.

Brood 3 resulted from a natural pairing in a cage between a male from the same source as the others and a female from Pine Grove, PA from a wild-collected cocoon. Mortality in this brood occurred during the larval stadia.

Larva (Fig. 1): Color bluish as in pure *C. promethea*, but most with pale lateral stripes. Red and yellow scoli large, either weakly clavate or tapered toward apex, in a few individuals some of these scoli bifid. Two larvae in Brood 1 were supertuberculate (see Peigler, 1977).

Cocoon: Most with well-developed attachments to stem. Color brownish-gray to brilliant gold. Exhibiting considerable variation yet mostly intermediate between parent species.

Male (Fig. 2): Characters of pure *C. promethea* very dominant, having very dark ground color and only minimal gold suffusion beyond postmedian line. Most with a trace of discal mark in forewing, not in hindwing. Underside more intermediate but traits of *C. promethea* still predominating. Wing apices generally more falcate (pointed) than males of either parent species. Some specimens with sparse scaling on thorax and abdomen.

Female (Fig. 3): Antennae and spiracular pattern intermediate. Color in most specimens reddish like *C. promethea* but lighter, some almost orange. Very pronounced black along postmedian line and around discal marks, as in *C. promethea*, but with even more contrast with adjacent areas. Underside quite intermediate, not dissimilar to pure *C. angulifera*! Some of these are larger than any females, hybrid or purebred, in the genus which I have seen—the proverbial heterosis.

C. [angulifera ♂ X (*angulifera* ♂ X *securifera* ♀)♀] X *C. securifera* ♀

All *C. angulifera* stock originated at Clemson, Pickens Co., S.C. and *C. securifera* stock at Berkeley Co., S.C. and after hand-pairing, the female laid 110 ova, of which six hatched. One larva was reared on tuliptree to the pupal stage and a female emerged in July. She was mated to a male *C. angulifera* but the ova did not hatch.

Larva: The yellowish lateral stripe prominent. Colored scoli (red anterior ones, yellow caudal one) very small. Black scoli so minute as to be almost invisible.

Cocoon: Large and puffy as in pure *C. securifera*, having a long, thin peduncle. Color of silk very golden.

Female: Color light orange. Very close to pure *C. securifera* on upper- and undersides. Discal marks in all four wings large, more as in *C. angulifera*.

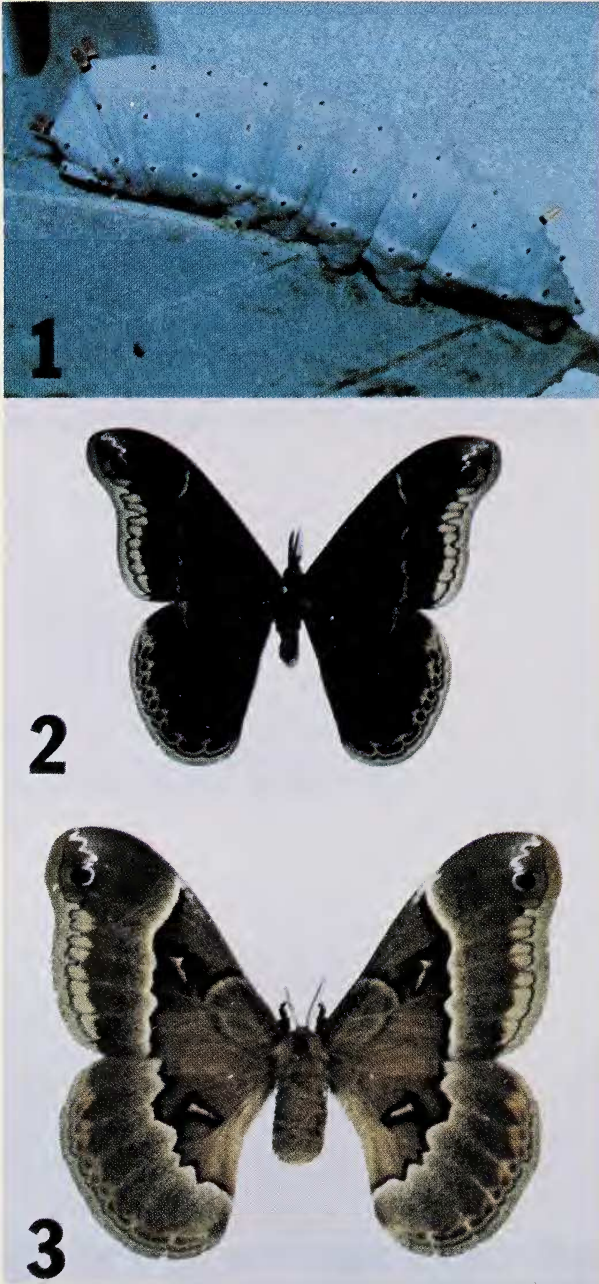


Fig. 1. Mature larva of *Callosamia securifera* ♂ X *C. promethea* ♀ from Brood 2. (Coloration in figure is too blue).

Fig. 2. Hybrid male of *C. securifera* ♂ X *C. promethea* ♀ from Brood 2.

Fig. 3. Hybrid female of *C. securifera* ♂ X *C. promethea* ♀ from Brood 1. (Coloration of actual specimen is more orange).

Conclusions from Above Crosses

The larvae of the three broods of *C. securifera* ♂ X *C. promethea* ♀ were all intermediate between parent species but exhibited pronounced differences between each brood (larvae of the pure species are rather constant). Larvae within a single brood showed minimal variation among themselves. Thus, each time a particular cross is repeated, one can expect similar but not identical results.

One of the conclusions from my earlier experiments (Peigler, 1977) was that hybrids involving *C. promethea* are sterile, i.e., cannot be backcrossed or make F₂ crosses. Due to different emergence dates for males and females and my being away when they emerged, I was unable to test the fertility of these moths by additional matings. Earlier crosses of *C. promethea* with the two other species failed to show two larval characters, which appeared in the new cross. One of these traits is the presence of a light lateral stripe, and the other is the whitish ring on the four red thoracic scoli just above the black bases. These two features can be seen clearly in the color photograph (Fig. 1) of one of the larvae, which could otherwise pass for pure *C. promethea*.

The three broods of the cross *C. securifera* ♂ X *C. promethea* ♀ gave similar results despite the fact that in one the female was from the South (from a population sympatric with *C. securifera*) and in the other two the females were from Pennsylvania (allopatric to *C. securifera*). This suggests that geographical genetic variation may be overshadowed by interspecific genetic differences. Many additional crosses are needed to develop such conclusions. Source localities of parental stock in hybridization studies should always be recorded.

The complex cross described above involving *C. angulifera* and *C. securifera* gave evidence that a small amount of fertility can be retained in hybrids for at least three generations, notwithstanding the fact that eclosion becomes very low already in the first backcross or F₂ cross (this is item 7 on Table 2), as also shown in some of my earlier crosses. This sterility occurs even when the ovipositing female is purebred.

As I pointed out (Peigler, 1977), most mortality in hybrid broods occurs in very young larvae, suggesting a genetically based physiological disharmony. Because of the ever-present problem of disease, and the fact that broods of the pure species also suffer from the same, such "negative data" cannot be very useful. I have no quantitative data available for percent success in rearing the three pure species from eggs to adults, but the usual results are normally no better than in the F₁ hybrid broods. Workers using *Drosophila*, certain plants, etc. enjoy higher numbers of experimental subjects on which to make statistical analyses. My methods of recording and tabulating data from the hybridization experiments follow those prescribed by Robinson (1971) in the introductory chapter of his book. A summary of results of all F₁ crosses I have made thus far is given in Table 1.

Isolating Mechanisms

Collins and Tuskes (1979) provided a definitive study of isolating mechanisms in another genus of saturniid moths, but these were predominantly prezygotic, and postzygotic ones such as reported here are desirable to complete their study. The present study of *Callosamia* would be enhanced by prezygotic observations such as theirs. I have attracted males of *C. angulifera* and *C. securifera* to captive females of *C. promethea* which were emitting pheromone during the flight times of the other two species. To determine whether pheromone differences exist between the three species will require observations such as those of Collins and Tuskes (1979).

Priesner (1968) gave data to suggest that the pheromone of all three species of *Callosamia* may be the same, and demonstrated that *Hyalophora cecropia* (L.) and *C. promethea* have different pheromones, but there is a partial degree of interattractivity. Rau and Rau (1929) reported a male of *C. promethea* attracted to a female of *H. cecropia* which emitted pheromone at the normal time before dawn.

Harbich (1976) presented an array of postzygotic isolating mechanisms in sphingid moths remarkably parallel to those of *Callosamia*. General classifications of reproductive isolating mechanisms were tabulated by Dobzhansky (1970) and Littlejohn (1969). My Table 2 roughly follows the format of the latter, but is modified to fit *Callosamia* in particular.

In nature each of the isolating mechanisms enumerated in Table 2 are tested if the previous one fails. In captivity only items 1a and 2 can be circumvented by artificial methods, and we may assume that temporal isolation is probably the most important one operating in nature. Selection should be expected toward those highest on the list (Littlejohn, 1969). In *Callosamia* crosses it is possible to see examples of all isolating mechanisms in Table 2 excepting item 2. Even this one could be tested by placing captive females in large cages with a choice of plants for oviposition.

I am not aware of any *valid* reports of wild hybrids ever being found, but private and museum collections should be checked nonetheless. The erroneous report of a *C. promethea*-*C. angulifera* wild hybrid in the *News of the Lepidopterists' Society* (June 1975, p. 10) was based on the erroneous statement that these hybrids occur by Collins and Weast (1961). Although the artificial hybrids of known parentage are recognizable as having intermediate or combined traits, wild hybrids could be easily overlooked unless a search with the explicit intention of finding them was undertaken. The searcher must be familiar with the normal variation which occurs within each species. If the three species of *Callosamia* can be crossed so easily in captivity, surely the primary isolating mechanisms must occasionally fail in nature. Examples of how this could occur include the possibility of artificial lights (such as streetlights) causing a female of *C. promethea* to continue emitting pheromone after nightfall, thus attracting

Table 1: RESULTS OF BASIC F₁ CROSSES IN CALLOSAMIA*

CROSS	No. eggs eclosed/ no. eggs deposited	No. pupating	No. ♂	No. ♀	fast-slow ♀♀ development
<i>angulifera</i> ♂ X <i>promethea</i> ♀	-----	33	19	14	fast ♀♀
<i>angulifera</i> ♂ X <i>promethea</i> ♀	6/115	2	1	1	-----
<i>angulifera</i> ♂ X <i>securifera</i> ♀	ca. 125/150	56	25	28	fast ♀♀
<i>angulifera</i> ♂ X <i>securifera</i> ♀	14/17	12	7	4	-----
<i>promethea</i> ♂ X <i>securifera</i> ♀	155/175	ca. 20**	4	0	-----
<i>promethea</i> ♂ X <i>securifera</i> ♀	109+/235	31	23	8	slow ♀♀
<i>securifera</i> ♂ X <i>promethea</i> ♀	125/134	10	6	4	slow ♀♀
<i>securifera</i> ♂ X <i>promethea</i> ♀	almost 100% eclosion	32	19	13	slow ♀♀
<i>securifera</i> ♂ X <i>promethea</i> ♀	almost 100% eclosion	15	9	6	slow ♀♀
<i>securifera</i> ♂ X <i>angulifera</i> ♀	21/128	12	6	5	slow ♀♀
<i>angulifera</i> ♂ X <i>Samia cynthia</i> ♀	over 90% eclosion	10	4	0	-----

*Including results from Peigler (1977 and 1978).

**Less than half pupated within the 55 cocoons which were spun.

Note: The only basic F₁ cross combination which I have not had the opportunity to make is *promethea* ♂ X *angulifera* ♀.

Table 2: ISOLATING MECHANISMS IN CALLOSAMIA

1. Reduction or elimination of cross-mating
 - a. Temporal
 - b. Mechanical
2. Incorrect or poor choice of foodplant for oviposition
3. Zygotic mortality—eggs fail to hatch
4. Weak F₁ progeny
 - a. Larval inviability
 - b. Disruptive diapause of pupa (see Peigler, 1978)
 - c. Adults not vigorous or well-formed (see Peigler, 1977)
5. Differing developmental times for female hybrids
6. Incorrect temporal activity of hybrid adults
7. F₁ hybrid or backcross sterility—partial or complete

Note: Mechanism 1a is pre-mating, 1b-7 are post-mating; 1-2 are prezygotic, 3-7 are postzygotic

males of *C. angulifera*. Even if the situation just proposed were to occur, it would be additionally necessary for the female to have failed to mate with a male of *C. promethea* earlier, which is also unlikely. Another possibility for cross matings is the emergence of females of two species in close proximity (within several cm), and males mating with both when attracted to the first "calling" female.

Circadian temporal isolation (allochronic mating behavior) in *Callosamia* was finally clarified by Ferguson (1972). Stated simply, *C. securifera* flies (i.e., females emit pheromone and males respond) during midday hours, *C. promethea* flies in late afternoon, and *C. angulifera* flies after sundown but before midnight. Supposedly a margin of one hour or more falls between these flight times when no species is flying. Male and female F₁ hybrids of *Callosamia* are possibly at a disadvantage for timing of correct mating behavior (item 5 on Table 2). Some data for this are in Peigler (1977).

One area of worthwhile investigation would be comparison of exact flight times of all three species of *Callosamia* between sympatric and allopatric populations. Specifically, would *C. promethea* fly earlier and/or later in Cedar Rapids, Iowa where it is common but the other two species do not occur, than populations of *C. promethea* living among its congeners? Indeed Ferguson (1972) stated that *C. securifera* flies earlier in coastal South Carolina (sympatric with *C. promethea*) than in south-central Florida (allopatric to *C. promethea*). The flight times of these two diurnal species probably vary with atmospheric conditions (Collins and West, 1961), with population density (low population levels perhaps having a broader flight time to ensure all females are mated), and with latitude, since photoperiod varies with the latter. Lepidopterists in several states could make useful contributions by keeping careful and persistent records on the circadian behavior of these moths, and noting the aforementioned parameters. Some data are already in place (Rau and Rau, 1929; Toliver *et al.*, 1979). There appears to be no seasonal temporal isolation in *Callosamia*, as was given by Ferguson (1972) for two species of *Hyalophora* in southeastern Canada.

Mechanical isolation (incompatibility of genitalia) is virtually negligible between *C. securifera* and *C. angulifera*, but *C. promethea* has considerably larger genitalia in both sexes than its congeners. Should we then be surprised that the flight time of *C. promethea* falls *between* the other two? Shapiro (1978) and Dobzhansky (1970) gave some convincing arguments against the "lock and key" significance, but I believe that mechanical differences in *Callosamia* do play a role in reducing natural hybridization. I have discussed (Peigler, 1977 and 1978) how this difference also hinders artificial hybridization experiments. Male genitalia of the hybrids are intermediate in size and shape.

Information on foodplant specificity in this genus can be found elsewhere (Ferguson, 1972; Peigler, 1976; Feeny and Scriber, 1979).

Since tuliptree is known to be the best foodplant for hybrids, ovipositing females of *C. promethea* which had mated to one of the other species would likely select plants unsuitable for the hybrid larvae, since *C. promethea* is polyphagous. A female of *C. securifera* would oviposit on sweetbay, although tuliptree would be a better host for her offspring if she had mated to a nonconspecific male. Brood 1 of the cross *C. securifera* ♂ X *C. promethea* ♀ included a striking difference in size of adults between those reared on tuliptree and those on sweetbay. Slower growth rates were also seen when rearing on sweetbay the larvae of *C. angulifera* ♂ X *C. securifera* ♀ (Peigler, 1976). It is possible that tuliptree is the best foodplant for pure *C. promethea*, as shown by certain growth data given by Feeny and Scriber (1979). Since *C. angulifera* is monophagous on tuliptree, the foodplant difficulty would not occur in wild hybrid broods in which the mother was *C. angulifera*. An F₁ hybrid female of any given parentage in the genus may be no more likely to select the best foodplant (tuliptree) than a less optimal one. Such foodplant differences in closely related species of Lepidoptera can certainly be interpreted as an isolating mechanism.

Female hybrids showing both heterosis (as I mentioned under the cross *C. securifera* ♂ X *C. promethea* ♀) and reduced viability (as I mentioned under *C. promethea* ♂ X *C. securifera* ♀ in Peigler (1977)) were obtained in crosses of the genus *Colias* (Pieridae) by Grula and Taylor (1980). The latter authors attributed these differences between sexes in hybrid broods and between reciprocal crosses to the X-chromosome, which apparently contains most or all genes governing size, developmental rate, wing pigmentation, and wing color pattern. *Callosamia* has not been investigated cytogenetically, except for spermatogenesis in *C. promethea* (Robinson, 1971). Moths in the same subfamily (Saturniinae) as *Callosamia* may have the XX♂:XY♀ or XX♂:XO♀ type of sex determination, the presence of sex chromatin seemingly correlated with the latter (Gupta and Narang, 1980). It would be best to reserve speculation about the genetic implications of results from crosses within this genus until such basic cytogenetic information is available. Haldane's Rule (Robinson, 1971: 24; Dobzhansky, 1970: 333) that the heterogametic sex is rarer or has reduced viability in hybrid broods certainly appears to hold for *Callosamia*. The pronounced sexual dimorphism of these moths will make such studies all the more enticing, since the findings of Grula and Taylor (1980) suggest increased sexual dimorphism connotes increased genetic incompatibility in hybridization of Lepidoptera.

A conspicuous phenomenon which is well-illustrated by *Callosamia* hybrids is that of males emerging before or after females of a hybrid brood, frequently the females diapause and the males do not (see Table 1). This was also shown by Cocault *et al.* (1980) in a hybrid brood of the saturniids *Graellsia isabellae* (Graells) ♂ X *Actias luna* (L.) ♀. Oliver (1978 and 1979) found the phenomenon in his nymphalid hybrids as did Grula and Taylor

(1980) in their hybrids of *Colias*. The differing developmental rates of the sexes in hybrid Lepidoptera are here proposed as an isolating mechanism (item 5 in Table 2) because such would reduce frequency of F_2 and backcross matings when hybrid broods are produced in nature. Based on the few F_1 crosses made thus far in *Callosamia*, it appears that fast female development occurs in hybrid broods in which the father is *C. angulifera*, and slow female development is to be expected in broods fathered by *C. securifera* or *C. promethea*.

Discussion

Some workers have advocated that isolating mechanisms are the result of speciation, not the cause (Ehrlich and Raven, 1969) while others appear to take the opposing viewpoint (e.g., Bush, 1969). Lewontin (1974: 161) felt that isolating mechanisms arise as a result of genetic divergence due to allopatry, and then become selectively reinforced upon secondary contact. These matters were also ably reviewed by Futuyma and Mayer (1980) who concluded that the genetic and selective causes of reproductive isolation are still largely unknown. In *Callosamia* I would propose that the large amount of genetic incompatibility inferred from the hybrid crosses is strong evidence that the genomes of the three species differ considerably, but allozyme analysis would be desirable as proof. These and other isolating mechanisms were probably increased in number and intensity after speciation was complete. Once allochronic mating behavior became perfected (whatever the cause) to the point that cross matings were no longer significant in number, the other mechanisms would increase in number and intensity due to genetic divergence, the three species then being on separate paths of evolution. The postzygotic ones cannot be assumed, in my opinion, to have evolved to be relied upon when the prezygotic ones failed. They are merely coincidental, but are to be considered valid isolating mechanisms in that they reduce chances of hybrids being produced, or when produced will fail to pollute the genomes of the pure species. Although the evolutionary processes which result in speciation and in reproductive isolation are still poorly understood, the two processes must evidently go hand-in-hand to the extent that one is not necessarily the result of the other.

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